FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

Attorney's Docket No.: XI/P6155US0

U.S. Appln. No.: 09/194025

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR97/00897	22 MAY 1997	23 MAY 1996

Title of Invention
IMMORTAL AVIAN CELLS

Applicant(s) for DO/EO/US
BOUQUET, Jean-Francois et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SEBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A **copy** of the International Application as filed (35 U.S.C. 371 (c)(2))

   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☒ A **translation** of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))

   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments had NOT expired.
   d. ☒ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☐ An **oath** or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. To 16. Below concern document(s) or information included:

11. ☐ An **Information Disclosure Statement** under 37 C.F.R. 1.97 and 1.98.

12. ☐ An **Assignment** document for recording. A separate cover sheet in compliance with 37 C.F.R. 2.28 and 3.31 is included.

13. ☒ A **First preliminary amendment**.
    ☐ A Second or subsequent preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☐ Other:
    ☐ **Small** Entity Statement
    ☐
    ☐

☐ A copy of the Notification of Missing Requirements under 35 U.S.C. 371.

☐ In the event that a petition for extension of time is required to be submitted herewith, and in the event that a separate petition does not accompany this response, applicant hereby petitions under 37 CFR 1.136(a) for an extension of time of as many months as are required to render this submission timely. Any fee is authorized in 17(c).

Date: 19 November 1998

U.S. Application No. (if known, see 37 CFR 1.5)	International Application No. PCT/FR97/00897	Attorney's Docket No.: XI/P6155USO

17. ☒ The following fees are submitted:

	Calculations - PTO use only	
Basic National Fee (37 CFR 1.492 (a) (1)-(5);		
☒ Search Report has been prepared by the EPO or JPO ..... $ 840.00		
☐ International preliminary examination fee paid to USPTPO ..... $ 670.00		
☐ No Int'l Prelim. Exam. fee paid to USPTO but Int' l Search fee paid to USPTO ..... $ 760.00		
☐ Neither Int'l Prelim. Exam. fee nor Int'l Search fee paid to USPTO ..... $ 970.00		
☐ Int'l Prelim. Exam. fee paid to USPTO & all claims satisfied PCT Art. 33(2)-(4) ..... $ 96.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$ 840.00**	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 mos. ☐ 30 mos. from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number filed	Number extra	Rate		
Total claims	13 - 20 =		X $ 18.00	$	
Independent claims	04 - 03 =		X $ 78.00	$	
Multiple Dependent Claim(s) (if applicable)			+ $260.00	$	
TOTAL OF ABOVE CALCULATIONS =				**$ 840.00**	
Reduction of ½ for filing by **small** entity, if applicable. Small Entity Statement must also be filed.				$	
SUBTOTAL =				$ 840.00	
Processing fee of $130.00 for furnishing the **English translation** later than ☐ 20 mos. ☐ 30mos. From the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 840.00	
Fee for recording the enclosed **assignment**, accompanied by a cover sheet $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$ 840.00	
				Amount to be: Refunded	$
				Charged	$

a. ☒ A **check** in the amount of $ 840.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 12-0555 in the amount of $_____ to cover the above fees.

c. ☒ The Commissioner is hereby authorized to charge any additional fees required or credit overpayment to Deposit Account No. 12-0555.

Note: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be be filed and granted to restore the application to pending status.

Please address all correspondence to THOMAS P. SARRO at the address (below) of **Customer Number: 000881**

SEND ALL CORRESPONDENCE TO:

Larson & Taylor
Transpotomac Plaza
1199 North Fairfax Street, Suite 900
Alexandria, Virginia 22314-1437

Douglas E. Jackson
Signature

Name: Douglas E. Jackson
Registration: 28518

Date: 19 November 1998

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE Patent

In re application of **BOUQUET, et al.**)
New Application) Atty's Dckt: XI/P6155US0
Filed: On even date herewith) Application Branch
For: IMMORTAL AVIAN CELLS)

PRELIMINARY AMENDMENT

Hon. Assistant Commissioner of Patents
Washington, D.C. 20231

S I R:

Preliminary to the examination thereof, please amend the above-identified application as follows:

IN THE CLAIMS:

Claim 5, line 2, delete "one of Claims 1 to 4", and insert therefor --claim 4--.

Claim 9, line 1, delete "any one of Claims 5 to 8", and insert therefor --claim 5--.

Claim 10, line 1, delete "any one of Claims 5 to 9", and insert therefor --claim 5--.

Claim 11, line 1, delete "any one of Claim 5 to 10", and insert therefor --claim 5--.

Claim 12, line 1, delete "any one of claims 5 to 11", and insert therefor --claim 5--.

Claim 13, line 2, delete "any one of Claim 5 to 12", and insert therefor --claim 5--.

REMARKS

The above amendments are being made in order to place the application in better condition for examination and to reduce the filing fee.

Favorable consideration is respectfully requested.

Respectfully submitted,

Date: November 18, 1998

Douglas E. Jackson

By: Douglas E. Jackson
Registration No. 28518

LARSON & TAYLOR
1199 North Fairfax Street, Ste. 900 • Alexandria, Virginia 22314 • (703) 739-4900

IMMORTAL AVIAN CELLS

The present invention relates to avian cell lines and their derivatives.

It is not possible to establish cell lines spontaneously from organs taken from avian species, as can be done in the case of some organs derived from mammalian species.

The only available cell lines to date were obtained using the transforming properties of certain avian viruses which possess oncogenic properties, such as the retroviruses of the avian leukosis group or Marek's disease virus, or certain chemical molecules such as methylcholanthrene and diethylnitrosamine.

For the most part, these cell lines are considerably transformed, which renders them unsuitable for multiplying vaccine viruses.

Authors are adopting a novel approach which consists in introducing into cells a vector which does not exhibit any oncogenic character but which is able to integrate, into these cells, a gene which is selected for its capacity to induce immortalization.

The first tests were carried out using vectors which integrate avian retrovirus genes such as erbA and erbB.

French Patent Application FR-A-2 596 770 proposes an immortalization method in which a culture of avian or mammalian cells is infected with a vector or a system which, while not being oncogenic for the said cells, is able to integrate a gene selected from v-myb, v-ets and v-erbA into these cells. The AMV, E26 and XJ12 viruses, with this latter being a virus derivative of the AEV virus in which the oncogenic v-erB gene has been deleted, can be appropriate vectors.

In practice, while these tests made it possible to obtain established cell lines from cells of the haematopoietic cell line, they did not give the expected results in the case of chick embryo cells in adherent culture, such as fibroblasts or epithelial cells.

It was possible to obtain untransformed avian cell lines of the myeloblastoid type (blood cells) using the oncogene myb (International Patent Application WO91/18971).

In parallel, authors have proposed using the early t and T genes of the simian virus SV40 for immortalizing cells derived from different mammalian tissues (D.S. Neufeld et al., Molecular and Cellular Biology, August 1987, 2794-2802, O. Kellermann and F. Kelly, Differentiation 1986, 32 : 74-81 and French Patent Application FR-A-2 649 721).

For its part, French Patent Application FR-A-2 649 721 proposes a method of conditional immortalization which, it is claimed, can be used for any cell type and in any species, with the aim in this case being that of remedying the drawback of the high degree of specificity of the conventional approaches (limitation to particular species and/or to particular cell types): transformation of cells with a transforming virus (adenovirus, Epstein-Barr virus, certain papovaviruses such as the SV40 virus or polyoma virus; for example, the SV40 virus is indicated as only transforming rodent cells and human cells); transfection with constructs which contain a transforming gene which is linked to a viral promoter; transfection with a transforming gene which is linked to a cellular promoter. The choice of this patent application falls on a construct which combines a DNA fragment from the regulatory sequence of vimentin and a DNA fragment which encodes an immortalizing gene, which construct can be the T antigen of the SV40 virus under the control of the inducible promoter of vimentin. This document never mentions the avian species.

The actual use of such viral oncogenes has never been described in the avian species, apart from the use of the 12S form of the E1A protein of human adenovirus 5, which made it possible to immortalize quail epithelial cells (Guilhot et al. (1993), Oncogene 8 : 619-624).

Contrary to all expectations, the inventors succeeded in producing immortal, untransformed avian cell lines.

More generally, the inventors have found that it was possible to prepare immortal, untransformed avian cell lines which are resistant to apoptosis even from cells of avian tissues, that is to say from cells other than circulating blood cells or haematopoietic cells.

The present invention therefore relates to immortal, untransformed avian cells which are resistant to apoptosis and which derive, in particular, from avian tissues, that is to say from cells other than blood cells or haematopoietic cells, in particular fibroblasts and epithelial cells, for example from embryos.

The present invention more especially relates to an untransformed, immortal avian cell line which is selected from the group consisting of:

- cell line TDF-2A bcl-2, which is deposited in the CNCM (Collection Nationale de Cultures de Micro-organismes de l'Institut Pasteur [Pasteur Institute National Collection of Microorganism Cultures]) under reference number I-1709
- cell line TCF-4.10, which is deposited in the CNCM under reference number I-1710
- cell line TCF-4.10 bcl-2, which is deposited in the CNCM under reference number I-1711

bcl-2 denotes that the cells of the cell line functionally integrate the bcl-2 gene, which confers on them resistance to apoptosis (WO-A-93/20200, which is hereby incorporated by reference).

The invention naturally covers the cells which are derived from these cell lines. By this, it is to be understood that it is not only the cells as deposited in the CNCM under the indicated references which are covered but also the cells which constitute the progeny of these deposited cells, i.e., on the one hand, those which are obtained by simple multiplication and which may undergo mutations during these multiplications and, on the other hand, those which are obtained after

deliberate modification, which are then termed the derived cells, and, of course, also those which have undergone the two types of modification.

The invention therefore also covers the derived cells which are obtained by modifications of the above cells. These modifications may consist in:

- Inserting one or more expression cassettes, each of which comprises one or more nucleotide sequences encoding a molecule of industrial relevance, with these expression cassettes being able to produce this molecule following insertion into the cells of the invention. The skilled person is fully conversant with the technique. Molecules of industrial relevance which may be mentioned, in particular, are viral subunits of the peptide, protein or glycoprotein type, in particular for use in a vaccine or a diagnostic reagent, protein molecules such as hormones, etc.

- Chronically infecting with a virus which is able to multiply in these cells, for virus or vaccine production purposes, with or without prior modification of the sensitivity towards this virus. The infection may also not be chronic but carried out on a batch of cells which is selected for the viral multiplication.

(The modifications described below are to be understood as preferably and advantageously being combined with the preceding two types of modification).

- Introducing survival or anti-apoptotic genes other than bcl-2, such as the genes which encode the human adenovirus p19E1B (Rao et al. (1992), Proc. Natl. Acad. Sci. USA 89 : 7742-7746), the Epstein Barr virus LMP-1 (Gregory et al. (1991), Nature 349 : 612-614) and BHRF1 (Pearson et al. (1987), Virology 160 : 151-161), the herpes simplex virus ICP34.5 (Chou and Roizman (1992), Proc. Natl. Acad. Sci. USA 89 : 3266-3270) and the baculovirus p35 (Clem et al. (1991), Science 254 : 1388-1390) proteins in order to render these cell lines more resistant to the culture conditions, in particular for maintaining confluence.

- Overexpressing genes which are involved in controlling the cell cycle using vectors which are suitable for increasing the rate of proliferation. Thus, it has been demonstrated that, in certain cases, overexpressing cyclin-encoding genes leads to the cell cycle being shortened and therefore to the rate of proliferation being increased (Rosenberg et al. (1995), Oncogene 10 : 1501-1509; Quelle et al. (1993), Genes and Dev. 7 : 1559-1571).

- Modifying the viral sensitivity spectrum of the cell lines by integrating genes which encode receptors for the viruses of interest, with a view to multiplying these viruses.

Reference may be made to the mammalian species, where expression of the receptor for the measles virus (CD46) by murine cells, which are normally non-permissive for the virus, results in these cells becoming sensitive to this virus and being able to replicate it (Naniche et al. (1993), J. Virol. 67 : 6025-6032). The interest is, in particular, in rendering cells sensitive to a virus in order to produce the virus on these cells.

- Integrating oncogenes which are able to accelerate cell growth.

It is self-evident that the derived cells according to the invention may comprise one or more of the above-described modifications.

The invention also relates to a method for producing molecules of industrial relevance or viruses, which method comprises culturing the above-described cells.

The present invention is directed, in particular, towards producing molecules or viruses for creating diagnostic reagents or vaccines, or else towards producing molecules of therapeutic relevance.

The invention will now be described in more detail with the aid of embodiments which are taken by way of non-limiting examples and with reference to the attached drawing, in which:

- Figure 1 shows the structure of the vector pDAMT, which is used to prepare the cell line TDF-2A, and in which:

LTR: direct repeat sequence (long terminal repeat)
LTR: deleted LTR
MTI: murine metallothionein I promoter
SV40 T+t: SV40 early region
SV40: SV40 promoter

- Figure 2 shows the structure of the vector pphMT, which is used to prepare the cell line TCF-4.10, and in which:

LTR: direct repeat sequence (long terminal repeat)
phleo: gene for resistance to phleomycin
SV40pA: SV40 polyA
MTI: murine metallothionein I promoter
SV40 T+t: SV40 early region

EXAMPLE 1 = Production of the TDF-2A cell line

I. Description of its origin and its characteristics

1.1 Description of the vector employed : vector pDAMT

It comprises the SV40 virus early region (encodes the T and t antigens) (HindIII/BamHI fragment) (Fiers et al. (1978), Nature 273 : 113-120) under the control of the mouse metallothionein I promoter (EcoRI/BglII fragment with the BglII site being transformed into a HindIII site) (Durnam et al. (1980), Proc. Natl. Acad. Sci. USA 77 : 6511-6515 ; Brinster et al. (1982), Nature 296 : 39-42).

The EcoRI/EcoRI fragment containing this transcription unit, derived from the vector pMTSVneo (Peden et al. (1989), Exp. Cell. Res. 185 : 60-72), was inserted into the XbaI site of the vector pDA1 (Aubert et al. (1991), J. Cell. Biol. 113 : 497-506). This latter vector is essentially derived from the genome of the Rous sarcoma-associated virus 2 (RAV-2) following modification of the 3' LTR. Thus, the U3 region of the

RAV-2 3' LTR was deleted and linked to the R and U5 regions isolated from the Rous sarcoma-associated virus 1 (RAV-1) LTR. The vector also carries a transcription unit which contains the gene for resistance to neomycin under the control of the SV40 promoter derived from the vector pSV2neo (Southern and Berg (1982), J. Mol. Appl. Genet. 1 : 327-341). See Figure 1.

1.2. Establishment of the cell line and demonstration that it is immortalized.

Cells derived from 14-day Muscovy duck embryos were transfected with vector pDAMT using the dimethyl sulphoxide (DMSO) method described by Kawai and Nishizawa (1984), Mol. Cell. Biol. 4 : 1172-1174. The transfected cells are then selected by applying geneticin G418 (150 µg/ml) for 15 days. The resistant clones are then subcultured regularly at a rate of from 1 to 2 passages per week. After this 3-month period of active proliferation, the cells entered into a crisis period during which most of the cells died. After this period, which lasted approximately 2 months, several clones resumed active proliferation, suggesting that they had been immortalized.

The TDF-2A cell line is thus derived from 2 cultures.

It was studied in more depth.

The TDF-2A cells achieved 200 passages, that is approximately 460 generations, and were thus maintained continuously in culture for more than 600 days. By comparison, control cells, which are not expressing the SV40 virus early region, cannot be maintained in culture for more than 20 passages.

1.3. Proliferation characteristics.

The immortalized cells are cultured at 38°C, in a roller bottle, in a medium containing 6% 10 × HAM F-10, 4% 10 × 199 HANKS, from 2.95% to 4% tryptose broth phosphate, from 5.6% to 2.5% sodium bicarbonate, 0.1% 100 × vitamin BME, 3% foetal calf serum, from 5% to 1% kanamycin and from 0.5% to 1% vancomycin.

Under these conditions, their rate of doubling is once every 24 hours.

1.4. Expression of the T antigen.

It was verified, by means of indirect immunofluorescence or indirect immunophosphatase using an antibody which is specific for the T antigen (Pab 101 : Santa Cruz Biotechnology ref. sc147), that all the cells express the T antigen in their nucleus, indicating that they have all integrated the vector.

This integration was additionally demonstrated by means of Southern blotting. The genomic DNA of the immortalized fibroblasts was digested with the restriction enzymes XbaI and BstXI. Hybridization with a probe which was specific for the T antigen (1018 bp NdeI/NdeI fragment) verified that the transcription unit, which expressed the immortalizing gene and which was inserted into the TDF-2A cells, had not undergone any major rearrangements. This was indicated by the fact that the sizes of the hybridization fragments obtained were in accordance with the expected sizes.

1.5. Absence of tumorigenic capacity.

The immortalized cells do not exhibit any tumorigenic capacity. They are incapable of forming colonies in semi-solid medium or of forming tumours on hen or duck egg chorioallantoic membrane. They are also incapable of forming tumours on nude mice, and on one-day old SPF (pathogen-free) ducklings and chicks.

1.6. Karyotype.

The karyotype of the TDF-2A cells was studied at the 114th and 135th passages. This verified that the cells were indeed of avian origin, with the microchromosomes characteristic of this species being present. Furthermore, the chromosomes which were observed are representative of the chromosomes which are encountered in primary duck embryo cells, thereby confirming the origin of the cell line.

II. Properties.

The TDF-2A cells exhibit, in particular, a sensitivity to the duck-specific viruses, such as

adenovirus, parvovirus and reovirus, which are customarily replicated on primary duck embryo cells. These viruses can therefore be produced on this cell line.

EXAMPLE 2 : Characterization of the TDF-2A cell line by identifying the integration sites.

The genomic DNA of the TDF-2A cells, which was prepared from cells derived from the 114th and 135th passages, was digested with the restriction enzymes BglII and KpnI. The DNA, which had been treated in this way, was then subjected to gel electrophoresis, followed by transfer to a nylon membrane; it was then hybridized with a probe which was specific for the T antigen (1018 bp NdeI/NdeI fragment). For example, digestion with BglII gives rise to two hybridization bands of large size (approximately 15 and 23 kb), suggesting the existence of two integration sites. Digestion with KpnI gives rise to one major band of large size (approximately 20 kb) and to at least one minor band, thereby confirming the existence of at least two integration sites.

EXAMPLE 3 : Production of the TCF-4.10 cell line

1. Description of its origin and its characteristics

1.1. Description of the vector employed: vector pphMT

It comprises the SV40 virus early region (encodes the T and t antigens) (HindIII/BamHI fragment) (Fiers et al. (1978), Nature 273: 113-120) under the control of the mouse metallothionein I promoter (EcoRI/BglII fragment with the BglII site being transformed into a HindIII site) (Durnam et al. (1980), Proc. Natl. Acad. Sci. USA 77: 6511-6515; Brinster et al. (1982), Nature 296: 39-42).

The EcoRI/EcoRI fragment containing this transcription unit, derived from the vector pMTSVneo (Peden et al. (1989), Exp. Cell. Res. 185: 60-72), was inserted into the EcoRI site of the vector pUT507

(marketed by CAYLA-FRANCE), being located 3' of the region for expressing the gene for resistance to phleomycin (Figure 2). The structure of vector pUT507 is described in Mulsant et al. (1988), Somatic Cell and Molecular genetics 14: 243-252.

1.2. Establishment of the cell line and demonstration that it is immortalized.

Fibroblasts derived from chick embryos were transfected with vector pphMT using the dimethyl sulphoxide (DMSO) method described by Kawai and Nishizawa (1984), Mol. Cell. Biol. 4: 1172-1174. The transfected cells are then selected by progressively applying (from 10 µg/ml to 50 µg/ml) phleomycin for 15 days. The resistant clones are then subcultured regularly at a rate of from 1 to 2 passages per week. After a period of active proliferation of approximately 2 months, the cells entered into a crisis period when cell growth is very weak and during which there is a very high rate of mortality. After a period which lasted from 3 to 4 months, a few cells of the TCF-4.10 clone resumed active proliferation, suggesting that they had been immortalized.

The TCF-4.10 cells thus achieved 200 passages in culture, that is approximately 400 generations, and were maintained in culture for 3 years. By comparison, control fibroblasts, which are not expressing the SV40 virus early region, cannot be maintained in culture for more than 20 to 30 passages.

1.3. Proliferation characteristics.

The immortalized fibroblasts are cultured at 38°C in a medium containing 6% 10 × HAM F-10, 4% 10 × 199 HANKS, from 2.95% to 4% tryptose broth phosphate, from 5.6% to 2.5% sodium bicarbonate, 0.1% 100 × vitamin BME, 3% foetal calf serum, from 5% to 1% kanamycin and from 0.5% to 1% vancomycin. Under these conditions, their rate of doubling is 0.7 times per 24 hours.

2.2. Expression of the T antigen.

It was verified, by means of indirect immunofluorescence or indirect immunophosphatase using an antibody which is specific for the T antigen (Pab 101: Santa Cruz Biotechnology ref. sc147), that all the cells express the T antigen in their nucleus, indicating that they have all integrated the vector.

2.3. Absence of tumorigenic capacity

The immortalized fibroblasts do not exhibit any tumorigenic capacity. They are incapable of forming tumours on hen or duck egg chorioallantoic membrane.

3. Properties.

The TCF-4.10 cells exhibit, in particular, a sensitivity to avian viruses. Viruses which may, in particular, be mentioned are the avian poxviruses, such as canarypox or fowlpox, or else the Marek's disease viruses (1, 2 and 3(HVT) serotypes) or the Gumboro disease virus. These viruses can therefore be produced on this cell line.

EXAMPLE 4: Multiplication of canarypox on TCF-4.10 cells.

The TCF-4.10 cells are seeded in a roller bottle. The canarypox is inoculated onto an established lawn. When the cytopathic effect engendered by the virus has become generalized, harvesting is carried out by shaking so as to detach the cell lawn. The harvested mixture therefore consists of the cell lawn and the culture supernatant. The whole is homogenized by treating with an Ultraturrax for 1 mín at 13,500 rpm (T25-type IKA appliance).

The infectious viral titre is determined by means of a micromethod carried out on a 96-well plate. The virus dilutions are inoculated onto a lawn prepared from secondary chick embryo cells. Each viral dilution is inoculated onto 6 wells. The plates are incubated in a CO_2 incubator for 8 days. The presence of the virus in the wells is checked by observing the characteristic cytopathic effect (CPE) under the microscope. The

infectious titre is calculated by the KARBER method and is expressed by the logarithm of the inverse of the viral dilution which gives 50% CPE [titre = d+r/Nx(n+N/2)], where d is the dilution expressed in logs when all the wells are positive, r is the dilution ratio, N is the number of wells per dilution and n is the number of positive wells between 0 and 100%.

Results: The viral titres obtained are equivalent to those obtained on primary duck embryo cells.

EXAMPLE 5: Integration of the bcl-2 gene

A vector which permits expression of the bcl-2 gene under the control of the CMV (human cytomegalovirus) promoter is transfected into the TDF-2A and TCF-4.10 cells using conventional transfection methods (DMSO method described by Kawai and Nishizawa (1984), Mol. Cell. Biol. 4: 1172-1174 or lipofectamine method in accordance with the supplier's (GIBCO-BRL) recommendations).

After the transfected cells have been selected, expression of the Bcl-2 protein is detected by Western blotting.

The cells which express the Bcl-2 protein are then tested for their ability to survive under culture conditions in which an apoptosis process is observed (maintenance of the cells at confluence).

Thus, in the case of the TDF-2A bcl-2 cells, the apoptosis process engendered by the cells arriving at confluence is deferred by from 3 to 4 days as compared with the TDF-2A cells. An increase in cell density at confluence is observed in the TCF-4.10 bcl-2 cells as compared with the TCF-4.10 cells.

CLAIMS

1. Avian cell line, which is immortalized, but untransformed, and resistant to apoptosis.

2. Avian cell line according to Claim 1, characterized in that it is obtained from cells of avian tissues.

3. Avian cell line according to Claim 2, characterized in that it is derived from fibroblasts or epithelial cells.

4. Immortal, untransformed avian cell line, which is selected from the group consisting of:

- cell line TDF-2A bcl-2, which is deposited in the CNCM (Collection Nationale de Cultures de Microorganismes de l'Institut Pasteur [Pasteur Institute National Collection of Microorganism Cultures]) under reference number I-1709.
- cell line TCF-4.10, which is deposited in the CNCM under reference number I-1710
- cell line TCF-4.10 bcl-2, which is deposited in the CNCM under reference number I-1711.

5. Immortal avian cells which are derived from the cell line according to one of Claims 1 to 4.

6. Cells according to Claim 5, characterized in that they contain at least one expression cassette which comprises at least one nucleotide sequence encoding a molecule of industrial relevance.

7. Cells according to Claim 6, characterized in that the nucleotide sequence encodes a viral subunit of the peptide, protein or glycoprotein type or encodes protein molecules such as hormones.

8. Cells according to Claim 5, characterized in that they are infected, preferably chronically, with a virus which is able to multiply in these cells.

9. Cells according to any one of Claims 5 to 8, characterized in that they contain a survival or anti-apoptotic gene other than bcl-2, which gene is preferably selected from the group consisting of p19E1B from human adenovirus, LMP-1 from Epstein Barr virus,

BHRF1 from Epstein Barr virus, ICP34.5 from herpes simplex virus and p35 from baculovirus.

10. Cells according to any one of claims 5 to 9, characterized in that they integrate vectors which are able to overexpress one or more of the genes involved in controlling the cell cycle in order to increase the rate of proliferation.

11. Cells according to any one of Claims 5 to 10, characterized in that they integrate genes which encode viral receptors.

12. Cells according to any one of Claims 5 to 11, characterized in that they integrate oncogenes which are able to accelerate cell growth.

13. Method for producing molecules of industrial relevance or viruses, comprising culturing cells according to any one of Claims 5 to 12.

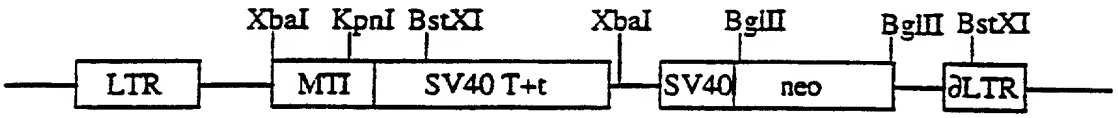
XbaI
KpnI
BstXI
XbaI
BglII
BglII
BstXI
LTR
MTI
SV40 T+t
SV40
neo
∂LTR

FIG. 1


LTR
phleo
SV40pA
MTI
SV40 T+t

FIG. 2

Mod 9606630

DECLARATION FOR USA PATENT APPLICATION

(including Design and National Stage PCT) Attorney's Docket ID: T

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below adjacent to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled "Immortal avian cells".

_____, the specification of which

(check one) ___ is attached hereto.

X is U.S. Application Serial No. 194,025 which was filed on November 19, 1998,

and (if applicable) amended on _____.

___ is International Application No. _____ which was filed on _____,

and (if applicable) amended on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).
I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s) (___ ADDITIONAL APPLICATIONS IDENTIFIED ON ATTACHED SHEET):

Number	Country	Day/Month/Year Filed	Priority Claimed Yes	No
96 06 630	FRANCE	23 May 1996	X	

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below; and insofar as the subject matter of each claim of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application in accordance with 37 C.F.R. 1.63(d). (___ ADDITIONAL APPLICATIONS IDENTIFIED ON ATTACHED SHEET.)

Application Serial No.	Day/Month/Year Filed	Status -- patented, pending, abandoned
PCT/FR97/0897	May 22, 1997	Pending

I hereby appoint the following to prosecute and transact all business in the Patent and Trademark Office connected therewith:

Andrew E. Taylor-17000	Thomas P. Sarro-19396	Harold L. Novick-26011	Mark J. Guttag-33057
Walter C. Gillis-22086	Ross F. Hunt, Jr.-24082	Douglas E. Jackson-28518	Daniel C. Mallery-33532
Marvin Petry-22752	William E. Jackson-24016	B. Aaron Schulman-31877	

Direct all telephone calls to _____ TEL (703) 920-7200.
Address all correspondence to LARSON & TAYLOR, 727 23rd Street South, Arlington, VA 22202; Facsimile Tel: (703) 892-8428.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of Sole or First Inventor	BOUQUET Jean-François	Citizenship	FRENCH
Post Office Address	40 chemin de l'hopital 69280 Ste CONSORCE. France.		
Residence - City, State (if different from P.O. address)	SAME AS ABOVE		FRX
SIGN AND DATE HERE	Inventor's Signature:	Date:	1.02.99
Full Name of Second Joint Inventor, if any	CLEUZIAT Catherine	Citizenship	FRENCH
Post Office Address	16 Rue de l'Espérance - 69003 LYON, FRANCE -		
Residence - City, State (if different from P.O. address)	SAME AS ABOVE		FRX
SIGN AND DATE HERE	Inventor's Signature:	Date:	1.02.99
Full Name of Third Joint Inventor, if any	SAMARUT Jacques	Citizenship	French
Post Office Address	169 bis Route de Genas 69100 VILLEURBANNE France		FRX
Residence - City, State (if different from P.O. address)	169 bis route de Genas 69100 Villeurbanne France		
SIGN AND DATE HERE	Inventor's Signature:	Date:	1.02.99

___ SEE ATTACHED SHEET FOR SIMILAR INFORMATION AND SIGNATURE FOR ADDITIONAL JOINT INVENTORS.

Date 1.02.99

Third Inventor DESPETTRE Philippe — JJ
First Name Middle Initial Last Name

Residence Le Treuil - 35, chemin de la Vernique 69130 ECULLY France FRX

Signature

Post Office Address Le Treuil - 35, chemin de la Vernique 69130 ECULLY France

Citizenship French

Date

Fourth Inventor
First Name Middle Initial Last Name

Residence

Signature

Post Office Address

Citizenship

Date

Fifth Inventor
First Name Middle Initial Last Name

Residence

Signature

Post Office Address

Citizenship

Date

Sixth Inventor
First Name Middle Initial Last Name

Residence

Signature

Post Office Address

Citizenship

Date

Seventh Inventor
First Name Middle Initial Last Name

Residence

Signature

Post Office Address

Citizenship

Date

Eighth Inventor
First Name Middle Initial Last Name

Residence

Signature

Post Office Address

Citizenship